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Analysis of the introgression of *Solanum bulbocastanum* DNA into potato breeding lines

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Abstract Somatic hybrids have been obtained between potato and *Solanum bulbocastanum* PI 245310, a Mexican diploid (2n = 2x = 24) species. Through restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) analyses it was found that the somatic hybrids contain each chromosome of the diploid parent and that the synteny of RFLP markers noted with tomato, potato and *S. brevidens* is largely maintained in *S. bulbocastanum*. RFLP analyses of BC1 progeny of two different hybrids indicated that a substantial number of markers were either lost or were heterozygous, in marked contrast with results previously noted with *S. brevidens*. A RAPD map for all 12 chromosomes of *S. bulbocastanum* was prepared and marker transmission was followed in three BC2 populations. Results with chromosomes 3, 8 and 10 from these populations are compared.

Keywords Somatic hybrid · potato · *Solanum bulbocastanum* · RFLP · RAPD

Introduction

Many wild *Solanum* species related to potato (*S. tuberosum*) have novel disease resistances which may provide durable protection against pests and minimize the need for pesticide applications. Many of these species cannot be crossed with potato and alternative means to combine the DNA of the wild and domesticated species are required. Somatic hybridization has been useful in this regard. Somatic hybrids between *S. brevidens* and *S. tuberosum* have provided a new source of resistance to Potato Leaf Roll Virus and *Erwinia* soft rots (Helgeson et al. 1986; Austin et al. 1988). Resistance to Potato Virus Y (PVY) can be obtained from somatic hybrids of potato and *S. etuberosum* (Novy and Helgeson 1994). *S. commersonii* and potato have been hybridized to give a new resistance to bacterial wilt disease caused by *Ralstonia solanacearum* (Laferriere et al. 1999).

For the past few years new genotypes of *Phytophthora infestans*, the pathogen that causes late blight in potato, have caused significant damage to the potato industry (Fry and Goodwin 1997). Somatic hybrids of potato and *S. bulbocastanum*, a Mexican species that is highly resistant to late blight, are resistant to these new genotypes of the pathogen (Helgeson et al. 1998). Late blight resistance from *S. bulbocastanum*, if stably captured in potato breeding lines, could greatly reduce the need for pesticides. It is therefore important to demonstrate that the DNA initially combined with potato in the somatic hybrid can be passed on to subsequent generations.

We previously used potato and tomato molecular markers developed by Bonierbale et al. (1988) and Tanksley et al. (1992) to examine introgression of *S. brevidens* DNA into potato breeding lines. These analyses have been aided by the fact that synteny of tomato and potato RFLP markers appears to be largely conserved in *S. brevidens*. Thus it was possible to examine segregation of chromosomes in progeny of hybrids between *S. brevidens* and potato (Williams et al. 1993) and to develop RAPD maps that were useful for examining recombination in backcross populations in this series (McGrath et al. 1994, 1996). With *S. brevidens* + potato, and materials derived from these hybridizations, there was a strong tendency for homologous pairing of chromosomes in meiosis. In the first

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generation, at least four of the chromosome pairs (4, 8, 9 and 10) exhibited only homologous pairing. Thus the pentaploids derived from these crosses tended to have one whole set of *S. brevidens* chromosomes (Williams et al. 1993). *S. brevidens* is an inbreeding, non-tuber forming species. In contrast, *S. bulbocastanum* is an outbreeding species that forms tubers. It is of interest to determine if the synteny conservation that is observed between *S. brevidens*, tomato and potato is retained in *S. bulbocastanum*. In addition, it is of interest to determine if there is evidence for introgression of *S. bulbocastanum* DNA into progeny of crosses between potato and the *S. bulbocastanum*-derived materials.

Materials and methods

Plant material

Plants examined in these experiments were backcross (BC) 1 and 2 progenies descended from products of somatic fusion between *S. bulbocastanum* PI 243510 (2n = 2x = 24) and *S. tuberosum* PI 23900 (2n = 4x = 48) (Helgeson et al. 1998). The clones of *S. bulbocastanum* and *S. tuberosum* used in this study are designated PT29 and R4, respectively. The BC1 progenies were obtained from crosses between three different somatic hybrids (J101, J103 and J138) and the *S. tuberosum* cultivars Katahdin (KAT) and Atlantic (ATL). Subsequently, BC2 progenies were obtained from crosses between selected late-blight-resistant BC1 individuals, J101K6 and J101K27, and the potato cultivars Atlantic and Norland, respectively. A third progeny group (J103K7 × A89804-7) was provided by Dr. Joe Pavek, Aberdeen, Id.

DNA extraction

For RAPD analysis, DNA was extracted from single leaves of material grown in vitro, using the microprep method described by McGrath et al. (1994). A miniprep DNA extraction protocol was used when larger quantities of DNA were required for RFLP analysis (Fulton et al. 1995).

RAPD analyses

Amplification reactions were carried out in 25-µl reaction mixtures containing 10 mM TRIS-HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.01% gelatin, 100 µM of each dNTP, 200 nM primer (Operon, Alameda, Calif.), about 15 ng of template DNA and 1 U of Amplitaq DNA polymerase (Perkin Elmer, Foster City, Calif.) using a Perkin-Elmer Model 480 thermocycler. The cycling program consisted of a 2-min denaturation step at 94°C followed by three cycles of 94°C for 1 min, 35°C for 1 min, and 72°C for 2 min, followed by 32 cycles of 94°C for 30 s, 35°C for 30 s, 72°C for 1 min, and a final 5-min extension step at 72°C (McGrath et al. 1996). Amplification products were size-separated in a 1% Synergel (Diversified Biotech, Boston, Mass.)-0.6% agarose gel, stained with ethidium bromide and visualized on a UV transilluminator.

RFLP analyses

RFLP analysis on a group of 24 BC1 individuals was done with radiolabeled probes by the methods described by Williams et al. (1990). RFLP analyses on BC2 populations were done using non-radioactive hybridization and detection methods. DNA samples (10 µg) were digested with *Eco*RI, *Hin*dIII, *Eco*RV or *Dra*I at a enzyme/DNA ratio of 7 U/µg. The fragments were size-separated on 0.8% agarose gels and blotted onto Hybond-N+ nylon membranes (Amersham Pharmacia Biotech, Piscataway, N.J.). Non-radioactive hybridization and detection methods were employed, following protocols provided by the manufacturer using either the ECL, Gene Images or AlkPhos systems (Amersham Pharmacia Biotech), depending on the probe used. The probes used were chromosome-specific tomato genomic and cDNA probes (Bonierbale et al. 1988; Tanksley et al. 1992). Enzyme/probe combinations which were informative are listed in Fig. 1.

Mapping

Markers were mapped using the haploid function of MapMaker (Lander et al. 1987). Only markers specific to *S. bulbocastanum* were scored and included in the mapping data set. In using the haploid function for mapping in the BC2 we are assuming that there is a single copy only of each *S. bulbocastanum* chromosome in the BC1 parent, due to preferential pairing of the *S. bulbocastanum* chromosomes in the somatic hybrid. RAPD markers were grouped at an LOD of 3. Easily scored but unassigned markers were recoded (presence of marker recorded as absence and vice versa) to test whether any of these markers were segregating in repulsion to other synteny groups. Only markers that give readily distinguishable polymorphisms with potato were included in the maps.

Results

RFLP analysis of first backcross plants

Thirty-four chromosome-specific tomato RFLP probes were used to analyze 24 BC1 individuals obtained from two different somatic hybrids between *S. tuberosum* R4 and *S. bulbocastanum* PT29 (Fig. 2). The assumption of general marker synteny between potato and *S. bulbocastanum* is supported by RFLP analysis of BC2 progeny from a different somatic hybrid between potato and *S. bulbocastanum* PI 275187 (Brown et al. 1996). Thus, the markers analyzed in Fig. 2 are grouped and ordered according to their positions on the potato/tomato maps (Tanksley et al. 1992). All *S. bulbocastanum*-specific markers which are homozygous in PT29 are expected to be present in the BC1 individuals, assuming preferential pairing in the somatic hybrid. However, marker loss was extensive in both progenies analyzed. Sixty-eight (17%) and 103 (24%) of 408 possible markers were lost in the J101 × KAT and J138 × KAT progenies, respectively. Markers detected by 11 probes (TG449, TG34, TG56, CD64, TG115, TG128, TG272, TG35, TG404, TG52 and TG26) segregate 1:1 in the BC1 progeny, indicating that these markers may be heterozygous in *S. bulbocastanum* PT29. Additional marker loss may be due to intergenomic recombination between *S. tuberosum* and *S. bulbocastanum* chromosomes. Marker loss was more extensive in the J138 × KAT population than in the J101 × KAT population. Two markers, TG301 on chromosome 1 and TG130 on chromosome 3, do not segregate in the J101 × KAT population, but appear to segregate 1:1 in the J138 × KAT population. Differences in marker segregation patterns from different somatic hybrids have been observed earlier in hybrids between potato and *S. brevidens* (Williams et al. 1990) and may be due to deletion of chromosomal regions during callus culture and shoot regeneration in vitro.

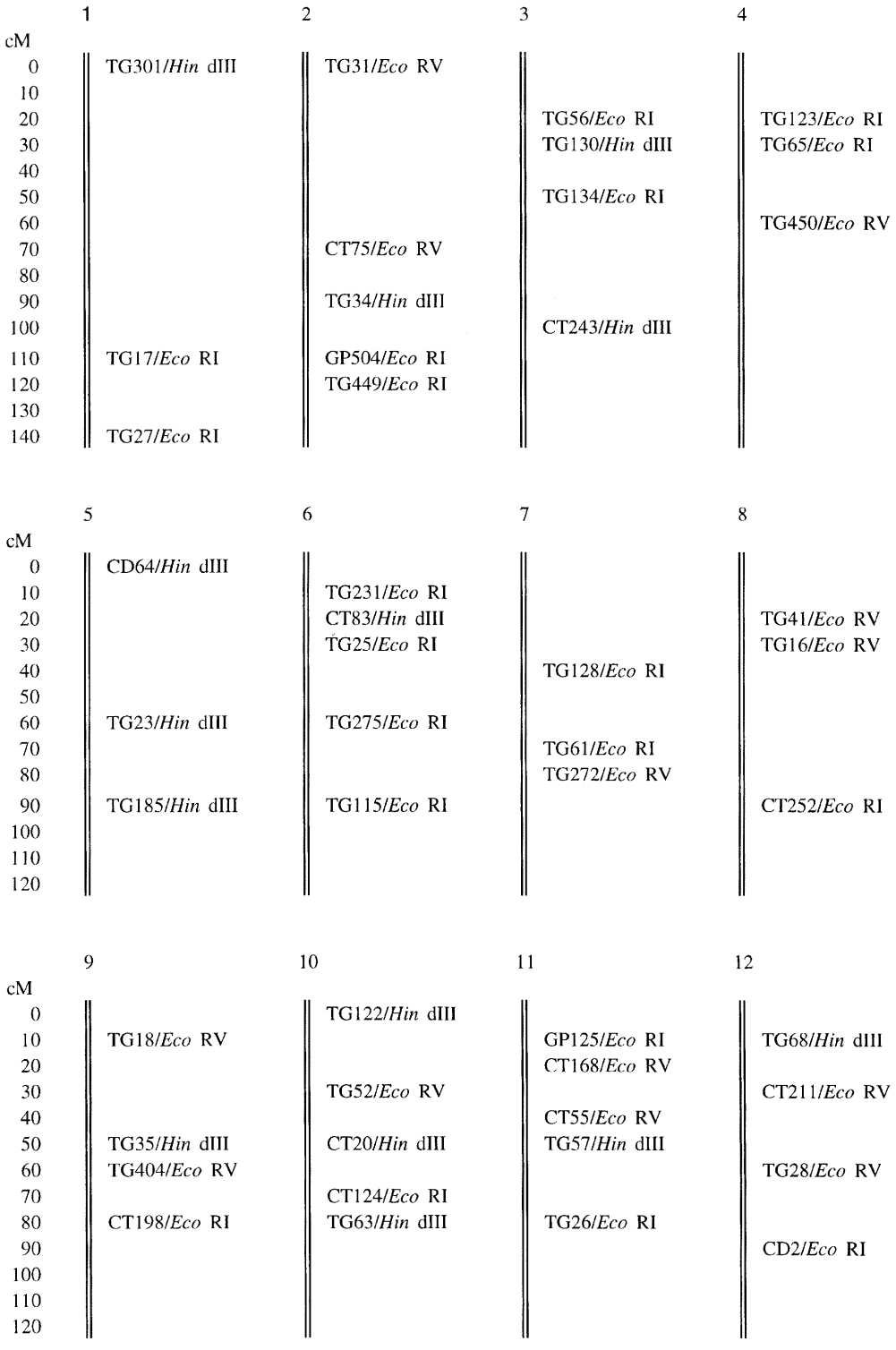

Fig. 1 RFLP probe/enzyme combination used for BC1 analysis and assignment of RAPD synteny groups to a chromosome. Note that the approximate marker position on each chromosome is based on the tomato map constructed by Tanksley et al. (1992)

RAPD synteny maps

Initially, 380 primers were screened for the production of useful markers polymorphic between *S. tuberosum* and *S. bulbocastanum*. Of these, 28% (106) provided strong *S. bulbocastanum*-specific markers for mapping in the three BC2 progenies examined: J101K27 × Norland (1K27 population, 50 individuals), J101K6 × Atlantic (1K6 population, 54 individuals) and J103K7 × A89804-7 (LB1 population, 69 individuals). Over 150 RAPD markers were scored in each of the three populations and assigned to 12 synteny groups. Marker synteny was conserved between the three populations examined, i.e., all easily scored RAPD markers common to the three populations mapped to the same linkage groups.

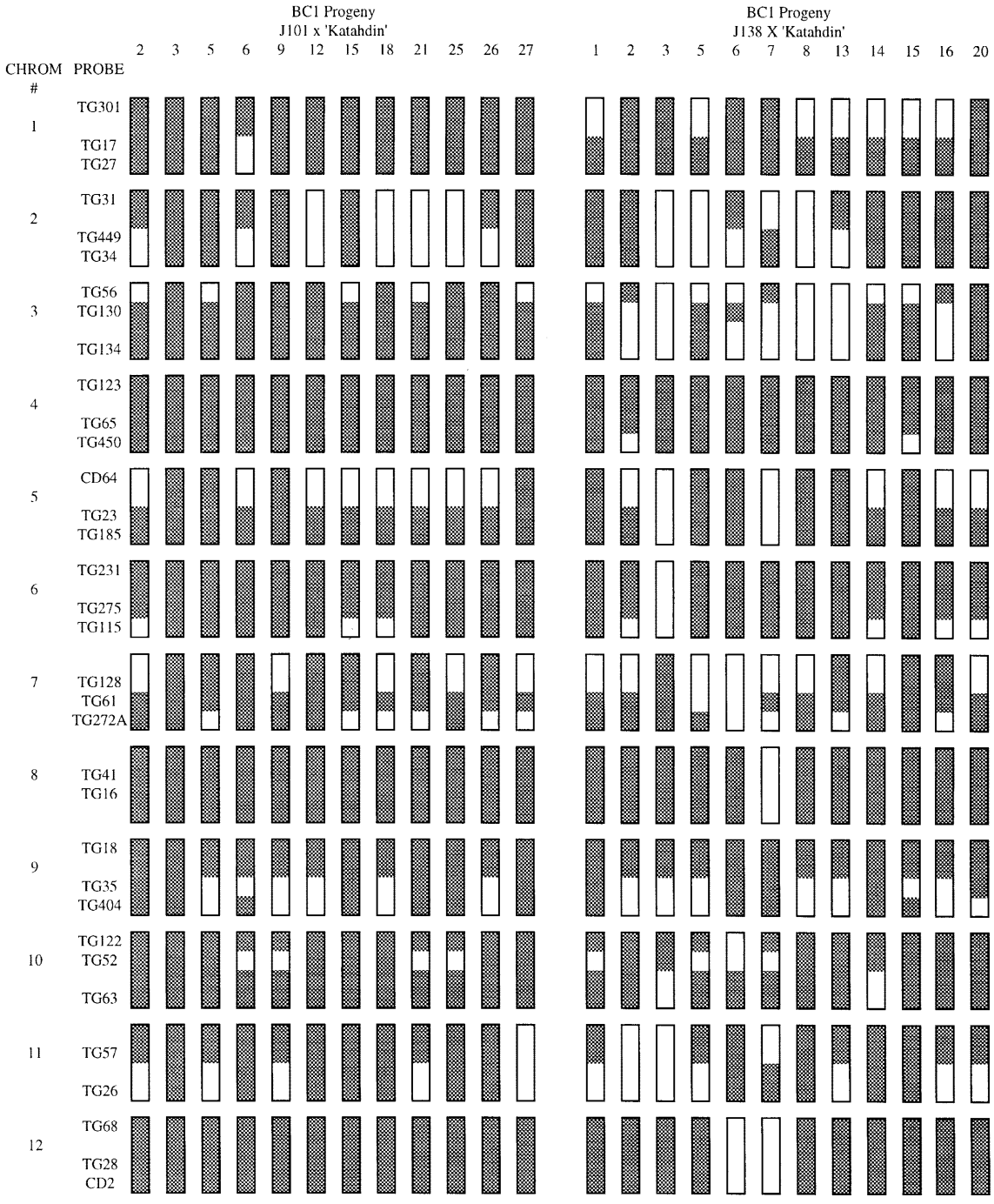

Fig. 2 Segregation of *Solanum bulbocastanum*-specific RFLP markers in 24 individuals from BC1 progenies of two different somatic hybrids between *S. bulbocastanum* PT29 and *S. tuberosum* R4. Marker order is based on potato and tomato maps (Tanksley et al. 1992). Marker presence is indicated by *shading*

To assign the RAPD synteny groups to chromosomes, eight BC2 individuals from the 1K27 population were chosen. These, as a group, gave a unique pattern of marker segregation for each 1K27 RAPD synteny group (Fig. 3). Cosegregation of 26 chromosome-specific RFLP markers with RAPD markers was used to assign chromosome numbers to the RAPD synteny groups. All the chosen 1K27 BC2 individuals lacked RFLP markers for tomato chromosome 11, as did the BC1 parent, J101K27. A RAPD synteny group found in the 1K6 and LB1 populations, but not in the 1K27 population, was identified as chromosome 11 with chromosome 11-specific RFLP probes. The RAPD synteny maps of all 12 chromosomes for the 1K6 population are shown in Fig. 4.

The three BC2 populations differed with respect to the presence or absence of synteny groups, individual RAPD markers segregating in the populations, and segregation ratios of markers common between the

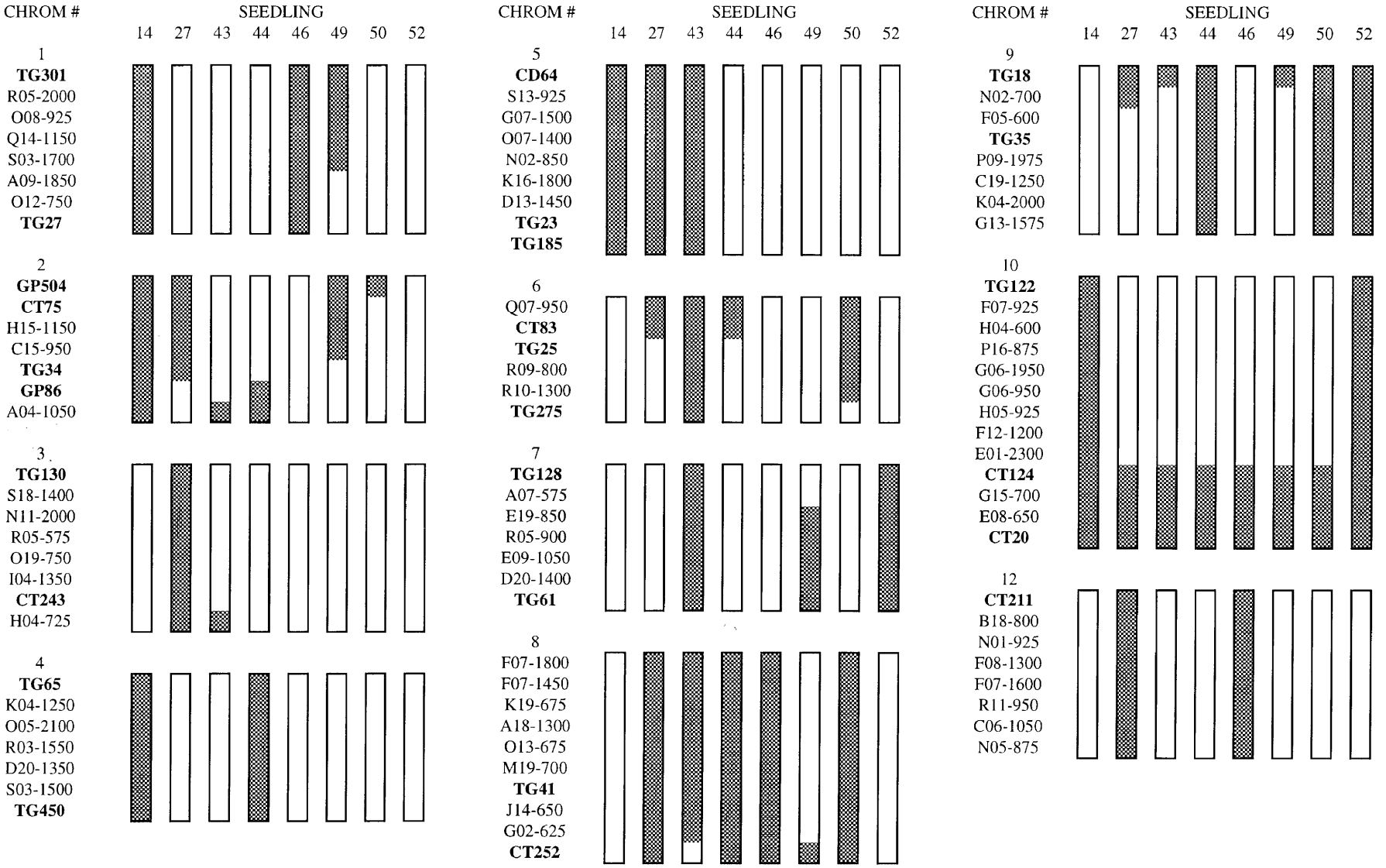

Fig. 3 Assignment of RAPD synteny groups to chromosomes based on cosegregation of chromosome-specific RFLP markers (in *bold*) with RAPD markers in eight BC2 individuals from the 1K27 × Norland population. No chromosome 11 markers were present in J101K27, the BC1 parent of this BC2 population. Marker presence indicated by *shading*

populations. The 1K27 population, as noted above, lacked all markers mapping to chromosome 11 in the other two populations. The LB1 population lacked markers mapping to chromosome 2 in the 1K27 and 1K6 populations. Only two RAPD markers have been assigned to this chromosome in the 1K6 population. Seedling J101K6, the BC1 parent of this population, may lack a portion of the *S. bulbocastanum* chromosome 2, as two of three chromosome 2-specific RFLP markers tested are missing from this individual (Fig. 2). Fully 25% of the markers scored, discounting the markers mapping to chromosomes 2 and 11, were absent from one or more of the three BC2 populations examined. These markers segregate in the BC1 and may be heterozygous in *S. bulbocastanum* PT29.

Marker transmission

The overall rate of marker transmission was 42% in the 1K27 and LB1 populations and 51% in the 1K6 population. Marker segregation did not deviate significantly ($p \geq 0.05$) from a 1:1 ratio for 60–80% of the markers scored. Most of the deviating markers were under-represented in the BC2 populations, which may be expected when following markers from a pentaploid parent. A few markers in each population – three each in the 1K27 and 1K6 populations and five in the LB1 population – segregated at ratios significantly greater than 1:1 ($p \leq 0.05$), indicating that these markers are probably present in more than one copy in the BC1 parent. If there was complete preferential pairing in the somatic hybrid, all *S. bulbocastanum*-specific markers would be hemizygous in the BC1 and segregate at a ratio of 1:1 or less in the BC2. Aberrant marker segregation ratios and synteny group differences between the BC2 populations are of interest as they indicate that some intergenomic recombination does occur in the somatic hybrid. Differences between populations were most pronounced for chromosomes 3, 8 and 10, and, for the purposes of illustration, these differences will be discussed in greater detail (Fig. 5). Differences between populations in the remaining linkage groups were less extensive.

Chromosome 3

A total of 26 different RAPD markers from the three populations group to chromosome 3 at an LOD of 3 or more. Four markers in this group differ between the populations: I04-1350 and M19-925 are missing from the LB1 population, E07-700 is missing from the 1K27 population and G13-1200 is specific to the LB1 population.

The 16 markers mapped on chromosome 3 in the 1K27 population all segregated at a ratio of 1:1 or less. In the LB1 population, chromosome 3-specific markers segregated at a ratio of 1:1 or slightly higher. In both populations these markers map to a single synteny group. In the 1K6 population, however, marker segregation ratios varied from slightly less than 1:1 to well over 1:1 ($p \leq 0.01$). These markers were grouped in two different synteny groups. The three terminal markers, N07-625, S18-1400 and L07-875, cosegregated and were present in 89% (47/53) of the progeny. These markers are most likely present in two unlinked copies in J101K6. E07-700 is linked to L07-875 in the LB1 population and is missing from J101K27. This marker is unassigned to a synteny group in the 1K6 population. However, the recoded marker (presence of marker scored as absence and vice versa) is assigned to chromosome 3. Thus E07-700 may be linked in repulsion to the PT29 homologue containing the majority of the chromosome 3 markers in this population.

Chromosome 8

The chromosome 8 synteny groups consist of 18, 17, and 13 markers, respectively, in the 1K27, 1K6 and LB1 populations. Ease of scoring varied among the populations and is reflected in the combination of markers successfully mapped. In the LB1 population a synteny group of only two markers, G02-575 and P09-550, segregates in repulsion to the chromosome 8 synteny group. The possibility of allelism between G02-625 and G02-575 was investigated in a diploid *S. bulbocastanum* population obtained from a cross between two individuals (*S. bulbocastanum* PI 243510 clone PT29 and PI 275187 clone SB22), in which both markers were present. Segregation ratios in a population of 137 F1 individuals confirmed that G02-625 and G02-575 are allelic or tightly linked in repulsion ($0.5 \leq p$ 1:2:1 ≤ 0.9) (Fig. 6). Portions of both PT29 chromosome 8 homologues must therefore be present in the LB1 population. This has since been confirmed by RFLP mapping using chromosome 8-specific probes which reveal codominant *S. bulbocastanum*-specific markers in our populations (data not shown).

Chromosome 10

Twenty-seven, twelve and seven markers in the 1K27, 1K6 and LB1 population, respectively, were grouped and assigned to chromosome 10. Four markers, F07-925, E01-2300, F12-1200 and G13-1000 vary among the three populations. Markers grouped into a single synteny group in the 1K6 and LB1 populations group to three different synteny groups in the 1K27 population. This is analagous to the situation for chromosome 3 in the 1K6 population. Markers E08-650 and G15-700 are found in over 90% of the 1K27 progeny (respectively 47 and 45/50) and are therefore probably present in two unlinked copies in J101K27. Markers K07-1100, Q11-1500 and Q11-1150 segregate 1:1 in repulsion to the bulk of chromosome 10 markers. The remaining markers segregate at ratios less than 1:1 (p 1:1 ≤ 0.05).

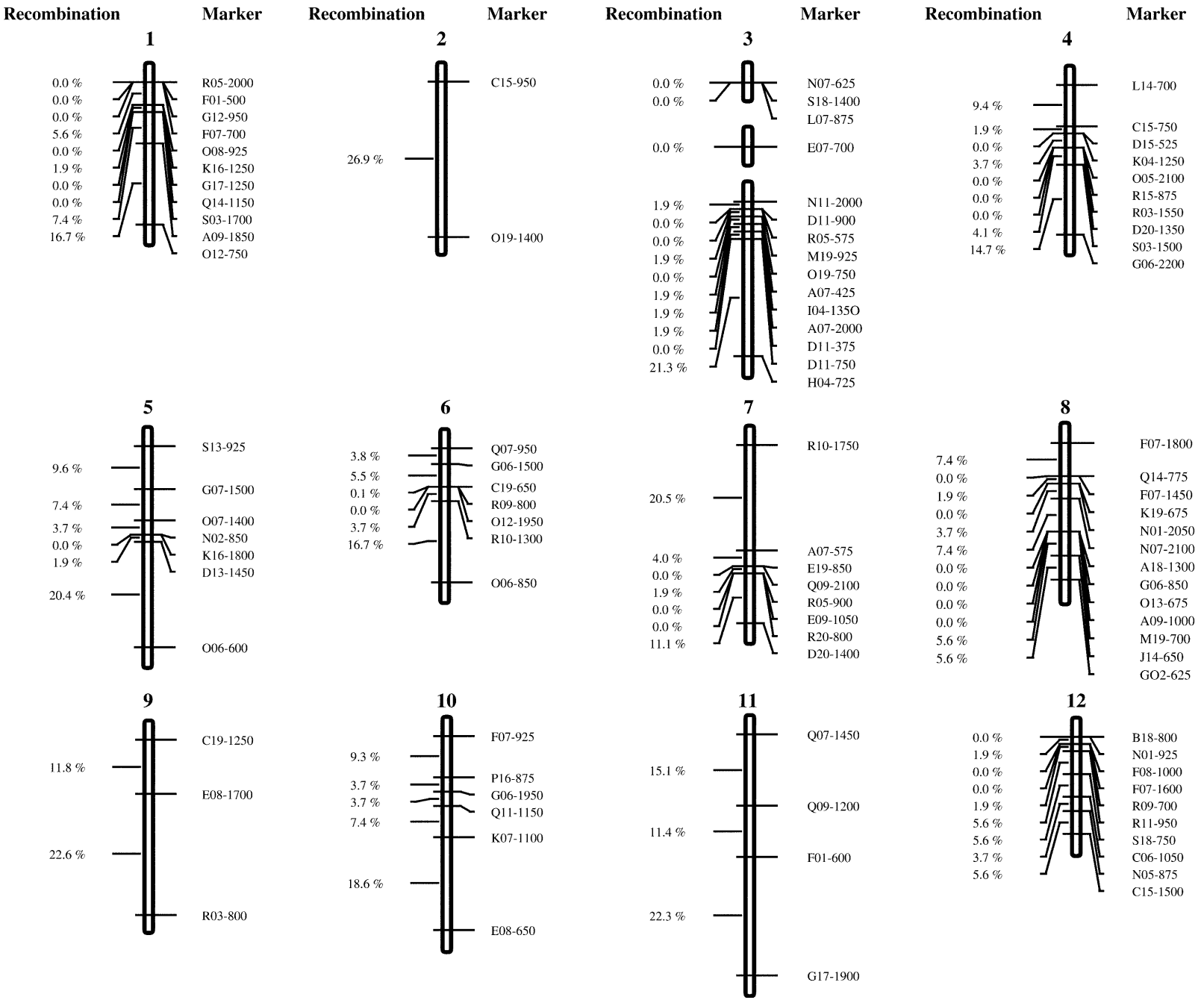
Recombination
Marker
1
0.0 % R05-2000
0.0 % F01-500
0.0 % G12-950
5.6 % F07-700
0.0 % O08-925
1.9 % K16-1250
0.0 % G17-1250
0.0 % Q14-1150
7.4 % S03-1700
16.7 % A09-1850
O12-750
2
C15-950
26.9 %
O19-1400
3
0.0 % N07-625
0.0 % S18-1400
L07-875
0.0 % E07-700
1.9 % N11-2000
0.0 % D11-900
0.0 % R05-575
1.9 % M19-925
0.0 % O19-750
1.9 % A07-425
1.9 % I04-135O
1.9 % A07-2000
0.0 % D11-375
21.3 % D11-750
H04-725
4
L14-700
9.4 %
1.9 % C15-750
0.0 % D15-525
3.7 % K04-1250
0.0 % O05-2100
0.0 % R15-875
0.0 % R03-1550
4.1 % D20-1350
14.7 % S03-1500
G06-2200
5
S13-925
9.6 %
G07-1500
7.4 %
3.7 % O07-1400
0.0 % N02-850
1.9 % K16-1800
D13-1450
20.4 %
O06-600
6
3.8 % Q07-950
G06-1500
5.5 %
0.1 % C19-650
0.0 % R09-800
3.7 % O12-1950
16.7 % R10-1300
O06-850
7
R10-1750
20.5 %
A07-575
4.0 % E19-850
0.0 % Q09-2100
1.9 % R05-900
0.0 % E09-1050
0.0 % R20-800
11.1 % D20-1400
8
F07-1800
7.4 %
0.0 % Q14-775
1.9 % F07-1450
0.0 % K19-675
3.7 % N01-2050
7.4 % N07-2100
0.0 % A18-1300
0.0 % G06-850
0.0 % O13-675
0.0 % A09-1000
5.6 % M19-700
5.6 % J14-650
GO2-625
9
C19-1250
11.8 %
E08-1700
22.6 %
R03-800
10
F07-925
9.3 %
P16-875
3.7 % G06-1950
3.7 % Q11-1150
7.4 %
K07-1100
18.6 %
E08-650
11
Q07-1450
15.1 %
Q09-1200
11.4 %
F01-600
22.3 %
G17-1900
12
0.0 % B18-800
1.9 % N01-925
0.0 % F08-1000
0.0 % F07-1600
1.9 % R09-700
5.6 % R11-950
5.6 % S18-750
3.7 % C06-1050
5.6 % N05-875
C15-1500

Fig. 4 RAPD synteny map based on 54 individuals from the 1K6 × Atlantic population. The marker name is given by the Operon primer code followed by the estimated marker size in bp. Chromosome 3 consists of several linkage groups

As mentioned earlier, a subset of the 1K27 BC2 population was used to assign RAPD synteny groups to chromosomes with the help of chromosome-specific RFLP markers (Fig. 3). Individuals non-recombinant within RAPD synteny groups were chosen for this purpose. However, since chromosome 10 in this population is composed of several RAPD synteny groups, we were able to orient the RAPD groups on the chromosome. Two long-arm specific RFLP markers, CT20 and CT124, cosegregate with RAPD marker E08-650, while the short-arm RFLP marker TG122 cosegregates with the cluster of RAPD markers including P16-875.

Marker transmission per seedling

Overall marker transmission to the individual seedling did not vary noticeably between the populations. On average, seedlings in the 1K27 and LB1 populations received all markers from three of 11 RAPD synteny groups, some markers from three additional synteny groups and no markers from six synteny groups (Table 1). Both J101K27 and the LB1 BC1 parent, J103K7, are missing a *S. bulbocastanum* RAPD synteny group. J101K6, on the other hand, has all 12 RAPD synteny groups and marker transmission was slightly higher in the 1K6 population. All markers from four groups and some markers from four additional groups were present on average.

For marker-selected breeding, the range of marker transmission present among seedlings is of greater interest. In each of the three populations some seedlings did not receive any complete *S. bulbocastanum* RAPD synteny groups from the BC1 parent. These seedlings were missing all markers from up to 10 RAPD synteny groups. At the other extreme, a seedling in the 1K6 population had all markers from nine synteny groups and at least some markers from the remaining three synteny groups.

Discussion

The rate of introgression of genes from one species into the genome of another can be greatly accelerated with the use of molecular markers (Young and Tanksley 1989). Once a linkage map including the trait of interest has been made, markers can be used both to track the trait and to select against genetic material that is unrelated to the trait of interest. Molecular linkage maps have been used extensively to locate traits of interest in potato, including genes and quantitative trait loci (QTLs) affecting resistance to late blight (Leonards-Schippers et al. 1994; El-Kharbotly et al. 1996; Li et al. 1998; Meyer et al. 1998).

Most of the mapping work reported here was done with *S. bulbocastanum*-specific RAPD marker information collected from three different BC2 populations. A key finding that can greatly aid in the development of markers is the demonstration that the synteny of a given marker group can be conserved in another species. Thus, as with *S. brevidens* (Williams et al. 1990; 1993), the synteny of RFLP markers in tomato and potato is retained in *S. bulbocastanum* (Brown et al. 1996). This greatly facilitated the assignment of RAPD synteny groups to putative chromosomes in the map developed here, and increases the utility of the map.

The RAPD map developed for the 1K6 population, comprised of 92 RAPD markers, covers 474 cM and is shorter than most RFLP maps of potato (Gebhardt et al. 1989, 1991) and an RFLP map of *S. bulbocastanum* (Brown et al. 1996). It is similar in size to the AFLP map of *S. bulbocastanum* (Rouppe Van der Voort et al. 1999). One problem encountered here, and by Grandillo and Tanksley (1996) with an interspecific tomato cross, is clustering of RAPD markers around the centromere regions of the chromosomes. Non-random distribution of RAPD markers has also been observed in other crops, including wheat (Kojima et al. 1998) and sugar beet (Nilsson et al. 1997). The clustering of RAPD markers in our population may be a factor that contributes to the reduced genome coverage of our maps relative to other interspecific potato RFLP maps.

Lack of intergenomic recombination and suppression of recombination in interspecific hybrids are serious obstacles to the successful introgression of desirable traits from wild species into crop plants. Loss of RFLP markers in BC1 progeny of somatic hybrids between *S. brevidens* and *S. tuberosum* indicated that some intergenomic recombination occurred in the hybrids, despite the fact that the species involved are only distantly related (Williams et al. 1993). Marker loss, at 20%, in BC1 progeny of somatic hybrids between *S. bulbocastanum* and potato was much more extensive than the 6% observed in BC1 progeny of somatic hybrids between *S. brevidens* and potato (Williams et al. 1993). However, most of the observed marker loss in the *S. bulbocastanum*-derived material was due to markers which segregated 1:1 in the BC1 progeny, indicating that these markers may be heterozygous in *S. bulbocastanum* PT29. It is therefore difficult to determine if marker loss is due to segregation of heterozygous PT29-specific alleles following homologous pairing or to cosegregation and loss of both alleles of homozygous PT29 loci following intergenomic recombination. *S. bulbocastanum,* an outcrossing species, is expected to be more heterozygous than *S. brevidens*, a self-pollinating species. In the *S. brevidens* BC1 progeny only 4% (2/55) of probes used detected heterozygous loci (Williams et al. 1993). In both cases the markers were codominant. In the *S. bulbocastanum* BC1 progeny 32% (11/34) of probes

1K27

Recombination	3	Marker
2.0 %		N07-625
0.0 %		S18-1400
4.0 %		L07-875
0.0 %		F13-1800
2.0 %		N11-2000
0.0 %		D15-1450
0.0 %		HO5-900
2.0 %		D11-900
4.0 %		R05-575
0.0 %		M19-925
0.0 %		O19-750
4.0 %		A07-425
0.0 %		I04-1350
0.0 %		A07-2000
0.0 %		D11-375
10.0 %		D11-750
		H04-725

Recombination	8	Marker
0.0 %		Q14-775
0.0 %		F07-1450
0.0 %		K19-675
0.0 %		N01-2050
4.0 %		G12-1200
0.0 %		A18-1300
0.0 %		G06-850
1.0 %		O13-675
1.0 %		A09-1000
2.0 %		L14-900
2.0 %		M19-700
10.0 %		O05-750
8.0 %		J14-650
		G02-625

Recombination	10	Marker
		F07-925
8.0 %		
2.0 %		H04-600
0.0 %		P16-875
0.0 %		G06-1950
0.0 %		G06-950
0.0 %		H05-925
2.0 %		F12-1200
		E01-2300
2.0 %		Q11-1150
10.2 %		Q11-1500
		K07-1100
4.0 %		G15-700
		E08-650

1K6

Recombination	3	Marker
0.0 %		N07-625
0.0 %		S18-1400
		L07-875
0.0 %		E07-700
1.9 %		N11-2000
0.0 %		D11-900
0.0 %		R05-575
1.9 %		M19-925
0.0 %		O19-750
1.9 %		A07-425
1.9 %		I04-135O
1.9 %		A07-2000
0.0 %		D11-375
21.3 %		D11-750
		H04-725

Recombination	8	Marker
		F07-1800
7.4 %		
0.0 %		Q14-775
1.9 %		F07-1450
0.0 %		K19-675
3.7 %		N01-2050
7.4 %		N07-2100
0.0 %		A18-1300
0.0 %		G06-850
0.0 %		O13-675
0.0 %		A09-1000
5.6 %		M19-700
5.6 %		J14-650
		GO2-625

Recombination	10	Marker
		F07-925
9.3 %		
		P16-875
3.7 %		G06-1950
3.7 %		Q11-1150
7.4 %		
		K07-1100
18.6 %		
		E08-650

LB1

Recombination	3	Marker
1.5 %		S18-1400
2.9 %		L07-875
5.8 %		E07-700
		N11-2000
14.5 %		
2.9 %		G13-1200
		O19-750
10.1 %		
		D11-375
23.8 %		
		H04-725

Recombination	8	Marker
		K19-675
11.6 %		
		N01-2050
4.4 %		G12-1200
4.4 %		A18-1300
2.9 %		G06-850
0.0 %		O13-675
0.0 %		M19-700
2.2 %		J14-650
2.2 %		G02-625

HOMOLOGUE

G02-575

15.4 %

P09-550

Recombination	10	Marker
		G13-1000
7.4 %		
0.0 %		P16-875
2.9 %		G06-1950
7.3 %		G15-700
		E08-650

Fig. 5 RAPD synteny group differences between BC2 populations 1K27, 1K6 and LB1. Chromosomes 3, 8 and 10 are illustrated. The marker name is given by the Operon primer code followed by the estimated marker size in bp. 1K6 chromosome 3 and 1K27 chromosome 10 are composed of several linkage groups. Presumed allelic markers are linked by a *dotted line*

used detected markers which segregated 1:1, but only four of these probes detected codominant markers for which both alleles could be followed in the progeny. Although the remaining probes also detected several restriction fragments in PT29, only one of the fragments was species specific. These markers need to be mapped in a diploid *S. bulbocastanum* population to determine whether or not they are in fact heterozygous in PT29. Brown did not find evidence for heterozygosity in a BC1 population derived from a fusion between a different *S. bulbocastanum* accession and potato (Masuelli et al. 1995).

Marker segregation data in our BC2 populations indicate that intergenomic recombination may occur in the somatic hybrids. Both marker segregation ratios significantly exceeding 1:1 and the presence of synteny groups segregating in repulsion to one another indicate that the markers involved are not hemizygous in the BC1 parent, as would be expected if chromosome pairing in the somatic hybrid were strictly preferential. Evidence for intergenomic chromosome pairing between more distantly related species has also been noted in somatic hybrids between *S. brevidens* and potato (Williams et al. 1993). However, further progeny testing revealed that some of the marker loss observed in BC2 progeny of somatic hybrids between *S. brevidens* and potato resulted from non-homologous intragenomic rather than homeologous intergenomic recombination (McGrath et al. 1996). Molecular marker analysis of BC3 generations and in situ fluorescent hybridization are currently being used to determine whether intragenomic non-homologous recombination also occurs in the progeny of *S. bulbocastanum* + potato somatic hybrids and to evaluate the extent of introgression of *S. bulbocastanum* DNA into the potato genome.

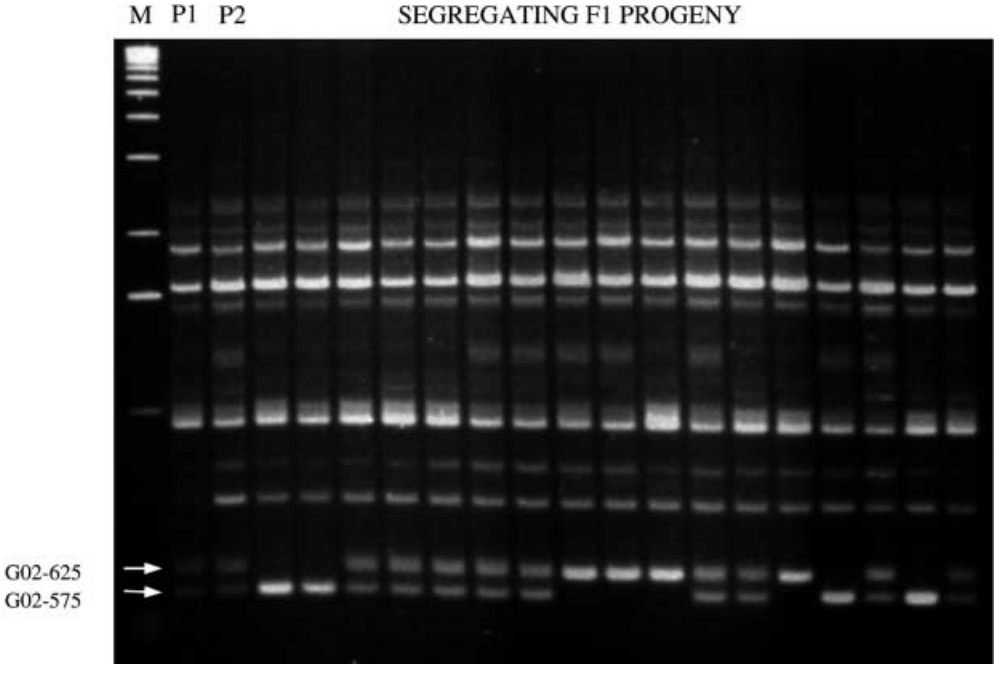

Fig. 6 Segregation of RAPD markers G02-575 and G02-625 in diploid *S. bulbocastanum* F1 progeny of a cross between two individuals heterozygous for the markers. M, 1-kb molecular ladder; P1, *S. bulbocastanum* PT29; P2, *S. bulbocastanum* SB22

Table 1 Transmission of *S. bulbocastanum*-specific RAPD synteny groups to BC2 progeny on a per seedling basis

Class	BC2 Population[a]					
	1K27[b]		1K6		LB1[b]	
	Average	Range	Average	Range	Average	Range
All markers present	3.1	0–7	4.0	0–9	3.1	0–8
At least one marker present	6.4	3–9	7.7	2–12	6.6	3–10
No markers present	5.6	3–9	4.3	0–10	5.4	2–9

[a] The values indicate the numbers of synteny groups found to satisfy the criteria listed in the first column in each BC2 individual tested
[b] The BC1 parents of these populations, J101K27 and J103K7 respectively, are missing a RAPD synteny group

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